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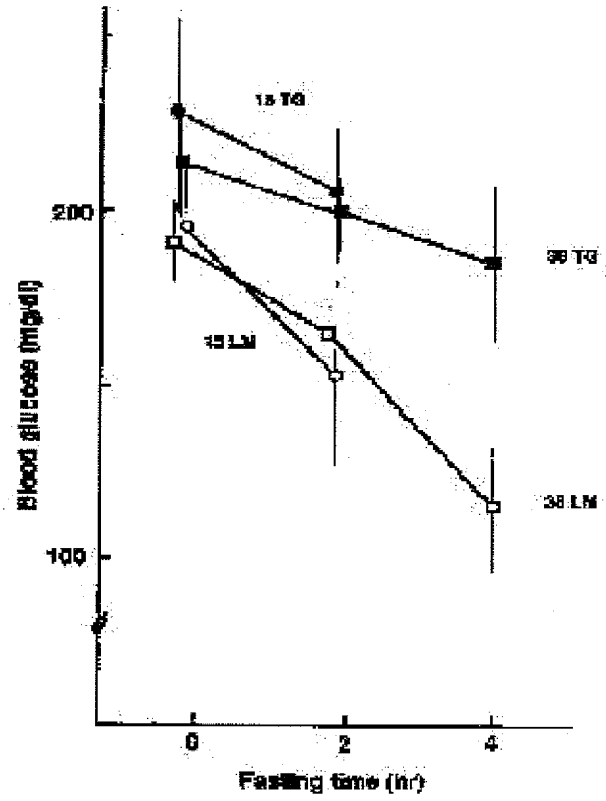
(54) GENE-INTRODUCED DIABETIC MODEL ANIMAL

(57)Abstract:
PURPOSE: To provide a disease model animal genetically exhibiting hyperglycemia or diabetes.
CONSTITUTION: A diabetes model mouse obtained by stably introducing an antisense glucokinase gene in chromosome. It is useful for the analysis of a blood sugar level maintaining mechanism and the development of an antidiabetic agent.



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CLAIMS

[Claim(s)]
[Claim 1] Transgenics mammalian which comes to introduce the gene which participates in blood sugar lifting into intracellular [which has totipotency].
[Claim 2] Transgenics mammalian according to claim 1 whose gene which participates in blood sugar lifting is a gene which controls glucokinase activity.
[Claim 3] Transgenics mammalian according to claim 2 whose glucokinase is specific to beta cells of pancreas.
[Claim 4] Transgenics mammalian according to claim 2 or 3 whose gene is an antisense glucokinase gene.
[Claim 5] Transgenics mammalian according to claim 4 which has the promotor, or the enhancer/promotor whom an antisense glucokinase gene commits in the organization or cell which is carrying out internality glucokinase gene expression.
[Claim 6] Transgenics mammalian according to claim 5 which has the promotor, or the enhancer/promotor who works by beta cells of pancreas.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the Homo sapiens animal used in disease modeling which has a diabetic genetic trait. This invention relates to a Homo sapiens animal used in disease modeling useful to research of the diabetes-mellitus onset device which comes to introduce the gene which participates in blood sugar lifting, for example, an antisense glucokinase gene, to intracellular [intracellular / which has the totipotency of mammalian], for example, a fertilized egg, the cure for diabetic, and development of a remedy in more detail.
[0002]
[Description of the Prior Art] If in charge of a cause break through of a Homo sapiens disease, a diagnosis and prevention, and development of treatment technique, the experiment system using a laboratory animal has played the important role. For this reason, it has by nature and hereditarily specific abnormalities similar to a Homo sapiens disease, and development of the animal used in disease modeling which generates that symptom at high rate serves as an important problem in a medicine area of research. Conventionally, such an animal used in disease modeling carries out whether it is obtained by being obtained by chance during breeding or inducing mutation artificially. However, by such approach, the animal used in disease modeling with a desirable characteristic can be obtained only by the very low probability. On the other hand, development of molecular biology in recent years and a gene engineering technique enables it to control the gene expression of a living thing artificially. The alteration of a characteristic is being attained also to a higher organism by the end of today. For example, since the transgenics (transgenic) animal which included the foreignness gene DNA in stability at a part of chromosome was reported by gar bosses [77 Proceedings of National Academy of Science (Proc.Natl.Acad.Sci.USA),

7380 pages, and 1980], various transgenic animals have been reported. These transgenic animals introduce a foreign gene DNA into the totipotency cell (a fertilized egg, an early embryo, embryonic stem cell) of an animal, return it to the oviduct or uterus of assumed parents, and are produced by making generating continue. thus -- since the obtained transgenic animal transmits a foreign gene also to a descendant -- a characteristic -- it is useful also as an amelioration animal. In this case, although a foreign gene will be discovered additionally, when a foreign gene makes it make it RNA which an internality gene makes discover a complementary antisense RNA, it is also possible to control work of an internality gene [Katsuki et al., 241 Science (Science), 593 pages, and 1988].

[0003] With change of eating habits or a living environment, a diabetic is increasing also in Japan and a break through of diabetic symptoms and amelioration of a cure are becoming a still more important theme in medicine today. Diabetes mellitus is divided into insulin dependence (IDDM, I-beam) and insulin non-dependence (NIDDM, II mold). When a viral infection etc. serves as a trigger and beta cells of pancreas are destroyed by the autoimmune reaction, the symptoms of IDDM are shown. However, it is NIDDM which has a diabetic generality and the insulin hyposecretion from peripheral insulin resistance and a beta cell is considered to participate in the onset. In blood sugar regulation, the beta-cell of islet is not enough solved [of what kind of environment the physiological function is governed and adjusted by the basis, and] yet, although the insulin which is the only blood sugar lowering hormone is secreted. Then, the transgenic animal incorporating the gene which carries out the code of the factor which raises blood sugar is considered to be useful as a Homo sapiens animal used in disease modeling which has a diabetic genetic trait. The gene which carries out the code of peptide hormone and cytokine, such as somatostatin which controls insulin secretion, galanin, and interleukin 1 (IL-1), as a factor which raises this blood sugar is mentioned. Moreover, the antisense gene which controls the activity of the gene which carries out the code of the protein sex factor which participates in transfer of an insulin secretion stimulus signal within beta cells of pancreas is mentioned. Furthermore, the antisense insulin receptor gene which controls an insulin operation, the gene which carries out the code of the abnormal insulin receptor are mentioned.

[0004] Although research is variously made about the regulator of this blood sugar level, specific glucokinase is discovered to the beta-cell of islet, and it is suggested recently that it has played the role important for sensing of the blood sugar level. Therefore, it is thought that manifestation control of the glucokinase of a beta cell is not only important for constant maintenance of a living body but deeply related to the origin of II type <2> diabetes mellitus. It was shown that the variation found out by the onset and the glucokinase

gene actually correlates by the family line of youth onset non-insulin dependent diabetes mellitus (MODY). Furthermore, this invention persons found out glucokinase gene variation in the frequent occurrence family line of Japanese adult onset non-insulin dependent diabetes mellitus [340 Lancet (Lancet), 1316 pages, and 1992]. Since all the patients to whom it is reported are in the condition of a hetero which has variation in one of the two of a gene, when the activity of glucokinase is halved in beta cells of pancreas, they have suggested that blood sugar goes up. in the patient with a glucokinase gene unusual [a still more important thing], the initial secretion reaction of an insulin in a carbohydrate tolerance test has received the failure -- it is -- lowering of this initial secretion reaction of an insulin -- a Japanese insulin non-dependence diabetic -- it is mostly found out by all.
[0005]
[Means for Solving the Problem] As a result of the old research on the above-mentioned diabetes mellitus, when the activity of glucokinase falls in an animal, it is thought that the same insulin secretion failure as a Japanese insulin non-dependence diabetic's generality is caused. However, the laboratory animal model in which lowering of glucokinase activity was shown until now was not obtained, and glucokinase activity and the relation of the onset of diabetes mellitus were not clear. Then, this invention persons attain the object by introducing the gene which participates in blood sugar lifting by producing the transgenic animal which considers producing a diabetes-mellitus onset model animal, for example, a mouse, therefore discovers the antisense RNA of glucokinase by beta cells of pancreas by introducing the gene which controls specifically the glucokinase activity in beta cells of pancreas. This invention namely, the gene to which (1) gene product (RNA is included) participates in blood sugar lifting The transgenics mammalian which it comes to introduce into intracellular [which has totipotency], and the gene which participates in (2) blood-sugar lifting The transgenics mammalian of the above-mentioned (1) publication which is the gene which controls glucokinase activity, and (3) glucokinases are specific to beta cells of pancreas. Transgenics mammalian the above (2) whose gene which controls the transgenics mammalian of the above-mentioned (2) publication and (4) glucokinases is an antisense glucokinase gene, or given in (3), and (5) antisense glucokinase gene The organization which is doing internality glucokinase gene expression Or the promotor, or the enhancer/promotor who works specifically or nonspecific by the transgenics mammalian of the above-mentioned (4) publication which has the promotor, or the enhancer/promotor who works specifically or nonspecific in a cell, and (6) beta cells of pancreas The gene which it has is related with the transgenics mammalian of the above-mentioned (5) publication which is an antisense glucokinase gene.

[0006] The cell which has totipotency here is a cell which has the capacity which can specialize in any organizations, and a fertilized egg, an early embryo, an embryonic stem cell, etc. are mentioned as mentioned above as these examples. The gene in which the activity of the factor which lowers the blood sugar level besides the gene which carries out the code of the factor which raises the blood sugar level as a gene which participates in blood sugar lifting is reduced can be mentioned, and specific glucokinase etc. is mentioned to the beta-cell of islet previously mentioned as a factor which lowers the blood sugar level. An antisense glucokinase gene is mentioned as a gene which controls this glucokinase activity. In addition, the gene which carries out the code of peptide hormone and cytokine, such as somatostatin which controls insulin secretion, galanin, and interleukin 1 (IL-1), as a gene which participates in blood sugar lifting is mentioned. Moreover, the antisense gene which controls the activity of the gene which carries out the code of the protein sex factor which participates in transfer of an insulin secretion stimulus signal within beta cells of pancreas is mentioned. Furthermore, the antisense insulin receptor gene which controls an insulin operation, the gene which carries out the code of the abnormal insulin receptor are mentioned. Antisense one and a glucokinase gene say a gene which discovers a complementary antisense RNA to RNA which an internality glucokinase gene makes, and the object is attained by building Glucokinase cDNA or its fragment into the reverse sense on a promotor's lower stream of a river. What was separated from the cDNA library obtained from a mouse, a rat, a guinea pig, Homo sapiens, etc. as glucokinase cDNA, the thing amplified and obtained by the PCR method, and the thing of composition or a semisynthesis are used, for example, the mouse beta cell origin glucokinase cDNA is mentioned as the example. As long as this cDNA fragment is the die length which complementary RNA is discovered [die length] to a part of glucokinase, and may decrease the activity, it may be a fragment of which part.

[0007] The gene to which the gene product of this invention participates in blood sugar lifting needs to have the required regulatory sequence, the promotor, and the enhancer, a gene is imprinted, and that by which RNA is produced as a result is pointed out. The promotor of these genes, the promotor whom a glucokinase gene originally has as an enhancer, and an enhancer are sufficient, and the different-species promotor who usually operates another gene expression, and a different-species enhancer can also be used. As these examples, an insulin promotor / enhancer, an amyrin promotor / enhancer, beta-actin promotor / enhancer, etc. are mentioned. It is desirable to have an enhancer further the promotor who works in said place, or if needed, and an insulin promotor is raised with the organization or cell which is carrying out internality gene expression, for example, pancreatic

islet beta intracellular, as the example.
[0008] Although a mouse, a rat, a guinea pig, a rabbit, a goat, etc. are mentioned as mammalian which introduces the gene of this invention, a mouse is preferably used from the ease on the training and handling, profitability, effectiveness, etc. As a method of introducing a gene, although the electric terebration, the liposome method, a calcium phosphate method, etc. can be used, the physical impregnation by the microinjection method of DNA to a fertilized egg etc. is desirable. A DNA impregnation cell is transplanted to the oviduct of assumed parents, cuts the tail head of the animal generated to the individual, extracts DNA in a somatic cell, and checks existence of an introductory gene by Southern blot analysis. The individual by which inclusion of the object gene was checked can tell a descendant this gene by mating. It is considered that what shows a 20 or more mg/dl high price as compared with normal values 160 - 200 mg/dl is effective as for the blood sugar level of these mammalians at any time. Among those, what went up in the range of 30 - 100 mg/dl is desirable.
[0009] When displaying a base by the code in this invention description and a drawing, it is IUPAC-IUB Commision on Biochemical Nomenclature. The following of the example is carried out based on the code to depend or the common use code in the field concerned.
DNA : cytosine RNA : Ribonucleic-acid mRNA: A part of base sequence may be embellished within RNA from which the antisense RNA which is a messenger RNA, and which is obtained by it in the antisense glucokinase gene of this invention is made by the internality glucokinase gene, and limits to which it can meet (addition, clearance, permutation to other bases, etc.). :deoxyribonucleic-acid cDNA: Complementary deoxyribonucleic acid A : Adenine T : Thymine G : Guanine C
[0010]
[Example] Although the following examples explain this invention more concretely, this invention is not limited to these. Transformant Escherichia coli HB one 101 obtained in the below-mentioned example (Escherichia coli) IGA-3 A stock is the trust number FERM on March 25, Heisei 5 to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, (FRI). It ****s as P-13553.
Example 1 What set respectively the end of the BamHI-NcoI and the human insulin promotor of the about 1.9 preparation kbs of an introductory gene [Sarvetnick et al., a cel (Cell), 52 volumes, 773 pages, and 1988] to SphI and BamHI using the linker was replaced with vector pKCR3for cDNA manifestation [Landais et al., 137 journal OBU immunology (J. Immunol.), 3002 pages, and the SphI-BamHI field in 1986. By the PCR method (Polymerase Chain Reaction), the mouse glucokinase cDNA fragment

amplified 283bp(s) from the 405th base to the 687th base, and was obtained from the cDNA library produced from the mouse beta cell stock MIN6 (Miyazaki et al., 127 endocrinology (Endocrinology), 126 pages, 1990). The used oligonucleotide primer is EcoRI in it. The following embellished so that a recognition sequence might be formed were used.
5 -- 'TGGGCGAATTCTACTTTGGA3' and 5 -- 'TCTGAGAATTCTGGGGTGGA3' -- the acquired PCR product -- EcoRI EcoRI of the rabbit beta globin gene sequence of the insulin promotor lower stream of a river of after digestion and an above-mentioned expression vector It introduced into the reverse sense to the site. In addition, the base sequence of the incorporated glucokinase cDNA was in agreement with what is already announced [Hughes et al., 266 Journal of Biological Chemistry (J. Biol.Chem.), 4521 pages, and 1991]. The ATG array of initiation is included in this array. This plasmid was introduced into 101 shares of Escherichia coli (E.coli) HB, and transformant E.coli HB101 IGA-3 (FERM P-13553) was obtained. In the antisense glucokinase gene introduced into the fertilized egg of a mouse, it is SphI about this plasmid. XhoI The cut-down straight chain-like DNA fragment of about 3.3 kbs was used. This introductory gene (transgene) is EcoRI-BglII which contains the poly A signal of the human insulin promotor's 3'5 of BamHI-EcoRI fragment [which contains the 2nd exon intron of a rabbit beta globin gene, and a part of 3rd exon at an edge] (640bp), and mouse beta cell origin glucokinase cDNA' side about 270 base pairs (reverse sense), and a rabbit beta globin gene as shown in drawing 1 . A fragment (523bp) is combined.
[0011] Example 2: The transgenic mouse which introduced the antisense glucokinase gene obtained in the production example 1 of a transgenic animal was produced as follows. The fertilized egg of the mating next day was extracted from the oviduct of a female mouse, and the above-mentioned DNA solution (5microg/(ml)) was poured into the male pronucleus of a fertilized egg using the thin glass pipet. You transplanted these fertilized eggs to 15-30 oviducts of a pseudopregnancy female mouse, and made it born by the natural birth or the cesarean section about 20 days after. 40 produced mice were bred, DNA was extracted from a part of tail by 4 weeks old, and existence of the introductory gene DNA was searched by the Sothern blotting methods. Consequently, it was checked that 14 animals are transgenic mice.
[0012] example 3: -- the analysis of the blood sugar level of a transgenic mouse -- these founder transgenic mouse was bred, after 5 weeks old, at any time, it collected blood from the tail and blood sugar was measured weekly using the blood sugar measuring instrument (ANTO sense: Miles Sankyo). Consequently, it was shown by the mouse with many copy numbers of Installation DNA compared with the mouse without an introductory gene

that blood sugar is intentionally expensive. Two (#15 and #38) in the transgenic mouse which showed the high blood sugar level were bred, and the second generation (F1) mouse was obtained. Among these, the blood sugar level of a male transgenic mouse [- (nine animals), ** (five animals)] and a control male mouse [O (nine animals), ** (five animals)] without a transgene was investigated. consequently, each when setting without giving food for 2 hours or 4 hours when food is given -- the direction of a transgenic mouse -- 20 - 30 mg/dl, 30 - 50 mg/dl, and 60 mg/dl -- the high blood sugar level was shown (drawing 2). In this result, the antisense glucokinase transgenics transgenic mouse shows that blood sugar is set highly hereditarily.

[0013]

[Effect of the Invention] By this invention, the gene which participates in blood sugar lifting is introduced into the cell which has totipotency, transgenics mammalian is produced, especially, an antisense glucokinase gene is incorporated to stability at a chromosome, and the animal used in disease modeling which presents hyperglycemia or diabetes mellitus hereditarily is offered. This diabetes-mellitus onset model mouse is the animal used in disease modeling which was extremely excellent also in the point considered to have the same initial secretion failure property of an insulin as a Japanese insulin non-dependence diabetic's generality. Therefore, this mouse is useful to the analysis of a blood sugar level maintenance device, or development of an antidiabetic drug.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The structure of the antisense glucokinase gene used for transgenic-mouse production is shown.
[Drawing 2] The blood sugar of the transgenic mouse (TG) of the male of the second generation (F1) of two transgenic mice (#15 and #38) which introduced the antisense glucokinase gene, and a control male mouse (LM) without a transgene is investigated. When food was given, blood sugar was measured using ANTO sense (Miles Sankyo) in each when setting without giving food for 2 hours or 4 hours.

[Translation done.]